Gynaecological malignancies in Lynch syndrome
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Chapter 7 | General discussion and future perspectives

GENERAL DISCUSSION

Lynch syndrome is an autosomal dominant cancer predisposition characterized by frequent and early occurrence of colorectal cancer and extra-colonic cancers. (1,2) Gynaecological consequences of Lynch syndrome (LS) are characterized by a higher risk and an earlier occurrence of the development of endometrial cancer and ovarian cancer than in the general population.

The overall lifetime risk of endometrial cancer for women with LS is 15-55%, with a mean age of the development of cancer at age 50-55 years depending on the gene mutation. (1-5) This is ten years earlier than the mean age at diagnosis of endometrial cancer in the general population (60-65 years). (6) In general endometrial cancer has a good prognosis as most cancers present at an early stage through symptoms of postmenopausal bleeding. In LS carriers, a substantial part of the endometrial cancers develop during perimenopausal age while irregular bleeding is common. (7-9) These perimenopausal women will benefit most of annual surveillance as irregular bleeding at that age is easily misinterpreted.

To early detect an endometrial (pre)malignancy in LS carriers, the current guideline for LS in the Netherlands advises annual endometrial surveillance from the age of 40-60 years by transvaginal ultrasound (TVU) measurement of the endometrial thickness and performing a standard endometrial sampling. (8-10) Only one study reported on patient acceptability of endometrial sampling in LS surveillance and they concluded that patient acceptability should form an integral part of studies that are evaluating the effectiveness of endometrial surveillance in women with LS. (11)

For ovarian cancer in women with LS, the overall lifetime risk varies between 6-12%. The mean age of women with sporadic ovarian cancer and with a BRCA2 gene mutation-related ovarian cancer is around 55-60 years of age and in BRCA1 mutation carriers around 45-50 years. In women with LS, ovarian cancer seems to occur approximately 15-20 years earlier than sporadic and BRCA2 gene mutation-related ovarian cancer (12-17) and 5-10 years earlier than in women with a BRCA1 gene mutation. (16,18-19) The mean age of ovarian cancer in women with Lynch syndrome is around 45 years. (20-26) Data about the outcome of ovarian cancer in LS are scarce as LS-associated ovarian cancer is a relatively rare disease. Some studies report a fairly good prognosis unlike sporadic and BRCA-related ovarian cancer, although it is unknown if this is the result of early detection through ovarian cancer surveillance during annual TVU,
a better response to chemotherapy or through different tumour characteristics compared to sporadic and *BRCA*-related ovarian cancer. Due to scarce data about efficacy of ovarian cancer screening in LS, it is difficult to formulate a surveillance advice for early ovarian cancer detection or prevention in women with LS. Therefore the current guideline only formulates to evaluate the ovaries while performing the TVU for endometrial surveillance. However, there is a lack of evidence of the effectiveness of TVU to detect ovarian cancer in LS and plenty of evidence that surveillance in *BRCA* carriers is not effective.\(^{(13,18,27-28)}\) Therefore, the guideline does not support annual ovarian surveillance alone in women with LS after hysterectomy. Besides, weighting pro’s and con’s, the guideline is hesitant to advice for preventive surgery before the age of 45 years.\(^{(10)}\)

**The main objectives of the studies in this thesis were:**

- To analyse the additional value of standard endometrial sampling to TVU, compared to endometrial sampling only in case of symptoms and/or enlarged endometrial thickness, during annual TVU in women with LS.
- To evaluate painfulness and patient acceptability of endometrial sampling and the effect on clinical decision making during the endometrial surveillance in women with LS and first degree relatives (FDR) at 50% risk of a gene mutation.
- To evaluate the feasibility of a potential less-painful alternative, to collect endometrial cells, by using a vaginal tampon, in women with LS and FDR.
- To examine the role of surveillance in early detection of ovarian cancer in women with LS.
- To analyse the clinical and histopathological characteristics of ovarian cancer in women with LS.
MAIN FINDINGS

PART I: DIAGNOSTIC ASPECTS OF ENDOMETRIAL SURVEILLANCE IN WOMEN WITH LS

In this thesis, the additional value of standard endometrial sampling to annual TVU was compared to endometrial sampling only, in case of symptoms and/or enlarged endometrial thickness in chapter two. Aim of the addition of endometrial sampling during annual surveillance in women with LS is to improve the effectiveness of endometrial screening in detecting more premalignant and early endometrial cancers and reduce interval carcinomas. In a study in 75 women with LS or FDR a 50% risk of LS, two different endometrial surveillance regimes in two time periods were compared.

During period I (from January 2003-December 2007), standard annual endometrial surveillance was performed by TVU and serum CA125 measurement. Endometrial sampling was performed only in symptomatic women, reporting irregular or postmenopausal bleeding, or in case of enlarged endometrial thickness, being > 12 mm in premenopausal women or > 4 mm in postmenopausal women.

In period II (January 2008-June 2012), annual endometrial surveillance by TVU (and CA125 measurement) was extended with endometrial sampling in all cases, irrespective of symptoms or TVU findings. The conclusion of this study was that the addition of standard endometrial sampling during annual TVU did not detect more early endometrial (pre)malignancies when compared to endometrial sampling only if indicated (chapter two). During both study periods all (pre)malignancies of the endometrium were found at an early stage and no interval endometrial cancers developed.

The painfulness and patient acceptability of endometrial sampling during single and repetitive procedures and the effect on clinical decision making during annual surveillance was measured in a prospective study (chapter three). In this study, 52 women with LS or FDRs of LS carriers who underwent repetitive annual gynaecological surveillance including endometrial sampling, and a cohort of 50 symptomatic women with postmenopausal or irregular bleeding without LS who underwent single endometrial sampling were included.
The level of pain during endometrial sampling in asymptomatic women with LS who underwent annual surveillance, as well as in symptomatic women with bleeding problems who underwent a single diagnostic endometrial sampling was measured. Pain intensity during endometrial sampling was registered with visual analogue scale (VAS) scores ranging from 0 (no pain) to 10 (unbearable pain). From this study it was concluded that performing an endometrial sampling is a painful procedure in all women who underwent this procedure. Irrespective of the indication, pain scores with a median VAS score of 5 (range 0-10) were reported. The level of pain did not aggravate in women with LS during subsequent annual procedures, although one in five decided to quit endometrial sampling and choose for an alternative. In this study, because of the pain, 4/50 (8%) women decided for annual surveillance with TVU alone, without standard endometrial sampling. Nine of 50 women (18%) decided for preventive surgery during the study period and out of these nine women, seven women reported pain as the main reason for this decision.

The feasibility of a potentially less-painful alternative for endometrial sampling was studied in chapter four. In this study 25 consecutive women with LS or FDRs of LS carriers who underwent repetitive annual gynaecological surveillance including standard endometrial sampling were asked to participate. Women who gave written informed consent were asked to insert a tampon vaginally 2-4 hours before the surveillance visit, which they were asked to remove at the surveillance visit.

The tampon was immersed in Thinprep preservation cytology solution® and thrown away afterwards. The solution was send to the pathology lab for cytological analysis. The tampon fluid was send for cytological analysis. Subsequently a TVU and endometrial sampling were performed. The endometrial sample was send independently for histological analysis, without mentioning that the patient participated in the tampon study. The quality of cells in both samples was compared as well as the yield. Pain intensity of both procedures was assessed with VAS scores.

Cyto-pathological examination showed that none of the 25 tampon samples contained endometrial cells, although all samples contained other vital cells (squamous epithelial cells and neutrophile granulocytes). Of the standard endometrial samplings 18/23 (78%) contained endometrial cells. In two women no endometrial sampling could be performed because the os internum of the cervix was closed (n=1) and one patient refused endometrial sampling because of fear for pain.
No endometrial (pre)malignancies were found in these 23 samples. The median reported VAS score of the tampon procedures was significantly lower compared to standard endometrial sampling: the median VAS score of the tampon procedure was 0 (range 0-10), while the median VAS score of the endometrial sampling was 5.5 (range 1-10; p<.001). It was concluded that although the tampon procedure was feasible and non-painful, apparently no endometrial cells were shed to the vagina in those asymptomatic LS carriers without endometrial pathology. In this study 78% of the standard endometrial samplings contained sufficient endometrial tissue for adequate histological diagnosis.

A limitation of this study was that it is still unclear if endometrial abnormalities will be found by intravaginal tampon procedures in symptomatic women with LS or in women with endometrial (pre)malignancies. Therefore more research has to be done to evaluate whether and how this female friendly surveillance by intravaginal tampons can be adjusted to be applicable in women who are under annual gynaecological surveillance because of LS.

**Does each surveillance visit of women with LS needs to be accompanied by endometrial sampling?**

In the study in chapter two of this thesis, no additional value was found with standard endometrial sampling compared to endometrial sampling only by indication, where other studies did. (8-9,29-30) Two other studies consisting of 175 and 100 women with LS who underwent gynaecological surveillance, found extra endometrial premalignancies during surveillance visits with endometrial sampling added to TVU, when compared to TVU alone. (8-9)

The reason for not finding more (pre)malignancies with adding endometrial sampling to all surveillance visits in our study might be that the surveillance interval was one year instead of two-three years as in the Renkonen study. (8)

Another explanation might be that the women in our study were younger, leading to less (pre)malignant events. The mean age of developing endometrial (pre) malignancies in our study, (chapter two) was 44 years (range 37-49 years) with all, except one, premenopausal women. The mean age of the women with an endometrial (pre)malignancy in the two largest studies of Renkonen et al. was 48 years (range 37-57 years) and of Gerritzen et al. 49 years (range 45-56 years). (8-9) These two studies reported more postmenopausal women compared to our study in chapter two. The
risk of developing endometrial cancer in women with LS is higher with increasing age, and therefore higher than in the younger population in the study described in chapter two. Another explanation might be that endometrial abnormalities in perimenopausal women (between age 40-50) are accompanied by irregular bleeding very often, although it is not always a symptom of a (pre)malignancy but can be a normal symptom of the hormonal imbalance during perimenopausal state.

In conclusion; in women with LS who undergo annual gynaecological surveillance, it can be discussed (shared decision) that a standard endometrial sampling can be omitted in pre- or postmenopausal women without any symptoms and a thin endometrium (< 4 mm), because the risk of an endometrial (pre) malignancy in this group is very low (overall risk around 1%). (31) In perimenopausal women, endometrial sampling should be offered as a standard procedure, because many of these women have irregular bleeding and the judgment of the endometrial thickness is hampered by lack of data at this age. Besides, irregular vaginal bleeding may be an early symptom of an endometrial (pre)malignancy.

Patients’ perspective on endometrial sampling
While performing annual gynaecological surveillance including standard endometrial sampling in women with LS, the clinical impression was that women often complained about the painfulness of the procedure, that this deteriorated over time, and that this was sometimes associated with fear for the procedure and even opting out. Therefore in chapter three, the level of pain during endometrial sampling was measured and pain scores compared between two groups of women who underwent either repetitive annual endometrial sampling because of LS, or single endometrial sampling because of symptoms.

Also the influence of pain scores on clinical decision-making during annual surveillance in women with LS was investigated (chapter three). Both groups (asymptomatic women with LS or symptomatic non-LS women) reported a median VAS score of 5 (0-10) during endometrial sampling. During subsequent procedures in women with LS, the median pain score did not aggravate, although one in five LS women chose an alternative for invasive annual endometrial sampling because of LS, or single endometrial sampling because of symptoms. In this study the pain scores of transvaginal ultrasonography were reported, which were associated with less discomfort than hysteroscopy or endometrial sampling. The authors concluded that transvaginal ultrasound would therefore be the most accepted test of choice for the majority of women during annual surveillance. (11)
Two studies reported about combined endometrial sampling and colonoscopy in women with LS under conscious sedation and concluded that this combination of surveillance under sedation is a less painful experience in women with LS than endometrial sampling in an office setting without sedation. (32-33)

**In conclusion;** endometrial sampling is a painful procedure irrespective of the indication and the need of this procedure. Therefore, the pro’s and cons of endometrial sampling in asymptomatic LS women with normal findings on the transvaginal ultrasound should be discussed with the patient and shared decision making is crucial. Shared decision making means that the doctor and the LS-carrier share information and build a consensus about the preferred surveillance for this particular woman in this situation. (34-35) If LS women have an indication for endometrial sampling (thickened endometrium or symptoms of irregular loss of blood) and refuse endometrial sampling because of pain, possible options for pain relief should be given. These options are using painkillers before the visit or performing gynaecological surveillance under local anaesthetics or conscious sedation.

**Is there an alternative for invasive endometrial sampling?**

In the feasibility study reported in **chapter four**, intravaginal tampons for 2-4 hours seem to be an acceptable approach with little if no pain during insertion and expulsion. All included women could insert a tampon vaginally at home, which was reported as an easy and (for all but one) painless procedure.

The aim of this study was to collect endometrial cells by intravaginal tampons. However, in 25 tampon procedures in asymptomatic women with LS, no endometrial cells were found, although all samples contained vital granulocytes and squamous epithelial cells. A few other studies reported about the feasibility and efficacy to collect endometrial cells by vaginal tampons. (36-39) In 2004 Fiegl et al. reported about a vaginal tampon study in symptomatic patients, 15 women with and 109 women without endometrial cancer. In this study DNA isolated from vaginal tampons was collected successfully for aberrant methylation of genes and was investigated before a hysterectomy was performed. Results of the vaginal samples were compared to the hysterectomy specimen and showed that DNA methylation in cells from women with endometrial cancer compared to women with benign endometrium was a very sensitive procedure to discriminate between women with and without endometrial cancer. (38) In 2015 a study by Bakkum-Gamez et al was published on data of vaginal tampons preoperatively in 38 women with endometrial cancer and 28 women who underwent a hysterectomy.
for benign reasons. DNA hypermethylation in endometrial cancer cells from the vaginal tampons was compared with tissue from invasive endometrial sampling.

The conclusion of both studies was that endometrial cancer could be detected by DNA hypermethylation in endometrial cell (fragments) obtained by transvaginal tampons. (38-39) In the tampon study reported in chapter four, no endometrial cells were found in the tampons in asymptomatic women and an obvious reason for the absence of endometrial cells in the tampon samples is most probably that these asymptomatic women without an endometrial (pre)malignancy were not shedding endometrial cells. All tampon samples contained vital cells however, which indicates that the collection and fixation procedure were sufficient.

**In conclusion;** the current Dutch guideline for LS advises annual endometrial surveillance by TVU and standard endometrial sampling from the age of 40-60 years. (10) In the study of chapter two no extra endometrial (pre)malignancies were found by adding standard endometrial sampling to the annual surveillance program in women with LS and the procedure was reported to be painful which is described in chapter three of this thesis.

In asymptomatic women with LS, the additional value of standard endometrial sampling seems to be limited. Annual follow-up in women with LS can be advised with or without omission of standard endometrial sampling after adequate counselling (shared decision) to plan an extra surveillance visit with endometrial sampling in case of symptoms as irregular or increased vaginal bleeding. In women who have in indication for endometrial sampling, who reported it as painful, options for adequate pain relieve should be advised and together with the woman a shared decision is made about the necessity for endometrial sampling and methods to relieve the pain during endometrial surveillance in women with LS. An alternative, less painful strategy such as vaginal tampons for endometrial analysis in women with LS is feasible (chapter four), however the efficacy in symptomatic women has never been studied and should be further evaluated.
PART II: CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF OVARIAN CANCER IN WOMEN WITH LS

The second part of this thesis is focused on LS-associated ovarian cancer. The clinical and histopathological characteristics of LS-associated ovarian cancer were evaluated and the role of surveillance in diagnosing these ovarian cancers was assessed. Until now, data about the characteristics of LS-associated ovarian cancer is scarce and the role of (annual) surveillance in early detection of LS-associated ovarian cancer is unknown. A few case reports and case studies suggest that these ovarian cancers might be different in clinical presentation and prognosis compared to sporadic and BRCA1/2 gene mutation-related ovarian cancers. (15,18,27,40-41)

The clinical and histopathological characteristics of LS-associated ovarian cancer. In chapter five the clinical and histopathological characteristics of ovarian cancer in women with LS were retrieved from a systematic literature review of studies published between 1979 and 2015. In this systematic review, 49 studies including data of 747 women with LS or first-degree relatives (FDR) who developed ovarian cancer were included. The quality of the assessed studies was analysed using the Newcastle-Ottawa Scale as recommended by the Cochrane Non-Randomized Studies Methods Working Group, and defined as high.

The reported mean age at diagnosis (available in 551 (73.8%) cases) of LS-associated ovarian cancers was 45.3 years with a wide age-range (19-82 years). The most frequent mutations (available in 548 (73.4%) patients) were MLH1 (38%) and MSH2 (47%). Histological data was available in 445 (59.6%) patients and the most frequent histopathological types were mixed type (mucinous/endometrioid/clearcell) carcinoma in 136 (31%) and endometrioid carcinoma in 103 (23%) patients. Stage at diagnosis was available for 426 (57.0%) patients, and was FIGO stage I/II in 281 (66%) with an estimated five year survival of 80-85%.

Comparable results were found in the Dutch cohort study in chapter six, which described women with LS-associated ovarian cancer in the Netherlands between 1966-2016 (chapter six). Data was collected from the Dutch LS registry (StOET) and the UMCG LS database. (42) In the Dutch LS registry, 46 LS-associated ovarian cancers were identified and 7 cases in the UMCG database, which were not already included in the Dutch LS registry.
The mean age of the 53 women at diagnosis of LS-associated ovarian cancer was 46.1 years with a wide age range (20-75 years). In these cohorts 4.9% of all LS-carriers had developed ovarian cancer by age 50 and 8.5% by age 70. The most frequently found gene mutations were MSH2 (32%) and MSH6 (28%). Endometrioid adenocarcinoma (n=21; 40%) and serous adenocarcinoma (n=19; 36%, 13 high grade and six low grade) were the most frequently reported histological types. Most LS-associated ovarian cancers (87%) were detected at an early stage (FIGO I/II) with an overall survival of 83% after a mean follow-up period 11 years.

What is the role of surveillance in early detection of LS-associated ovarian cancer?
In the systematic review described in **chapter five** data was analysed about the role of surveillance in early detection of ovarian cancer in women with LS. The number of cases that underwent gynaecological surveillance was scarce as only 6/49 studies in this analysis described ovarian surveillance in women with LS. Only 7/22 (32%) of the LS-associated ovarian cancers in these six studies were found during a surveillance visit. In the other 43 studies the remaining 725 LS-associated ovarian cancers were diagnosed without annual surveillance, mostly before the diagnosis of LS. Nevertheless, most ovarian cancers were detected at an early stage, suggesting that the role of surveillance was limited in the early detection.

In the cohort study described in **chapter six** consisting of 53 women with LS-associated ovarian cancer, a total of 41 of 53 (77%) patients were diagnosed with ovarian cancer before LS carrier status was known. 34/41 (83%) were diagnosed at FIGO stage I/II and seven (17%) patients with FIGO stage III. In 12/53 (23%) women ovarian cancer developed after starting annual gynaecological surveillance for LS. Of these 12 ovarian cancers detected in LS carriers under surveillance, one was an interval ovarian cancer (FIGO stage IC), three were diagnosed during gynaecological surveillance in asymptomatic women. The remaining 8/12 LS-associated ovarian cancers in women under surveillance were found in symptomatic women reporting irregular or postmenopausal bleeding. In six of these, ovarian cancer was detected following surgery for a (pre)malignancy of the endometrium. All 12 ovarian cancers in women under surveillance were early stage (FIGO stage I/II). There was no significant difference between the FIGO stages of the women who underwent annual surveillance and who did not. (p =0.31)

In conclusion; the early stage at diagnosis of LS-associated ovarian cancer could not be attributed to annual surveillance in these studies, as the rate of early stage disease was not significantly different from the non-surveyed patients. Besides, a substantial
part of the (early stage) ovarian cancers were diagnosed in symptomatic LS carriers with abnormal vaginal bleeding because of an endometrial (pre)malignancy for which they were operated.

What are the clinical differences between LS-associated ovarian cancer compared to sporadic and BRCA-related ovarian cancer?

After performing these two studies about ovarian cancer in women with LS it can be concluded that ovarian cancer in women with LS has a different clinical presentation and prognosis when compared to sporadic ovarian cancers and to ovarian cancer in women with a BRCA1/2 gene mutation, which could not be attributed to annual surveillance. Therefore, clinical differences might be due to biological differences.

1: Early age of onset
The mean age of onset of LS-associated ovarian cancer is 15-20 years earlier than the mean age at diagnosis in women with sporadic and BRCA2 gene mutation-related ovarian cancer (12-16,43) and 5-10 years earlier than the mean age at diagnosis of ovarian cancer in women with a BRCA1 gene mutation. (18-19,44) Besides, the age-range of LS-associated ovarian cancer is wider and especially a very early age of onset (< 35 years) is more common in LS than in sporadic or BRCA-associated cases. (13,18-19)

2: Other histological types
The distribution of histological types of LS-associated ovarian cancer is different from sporadic and BRCA cases. In the studies in this thesis 65-75% of the ovarian cancers were of non-serous type, while in sporadic and BRCA cases the majority of ovarian cancers are of the high-grade serous type. (13-14,18) The high-grade serous type ovarian carcinomas mostly derive from the distal end of the fallopian tube and non-invasive serous tubal intraepithelial carcinoma's (STICs) have been identified in prophylactic removed tubes in BRCA carriers. (45-46) Endometrioid and clear cell ovarian cancers are associated with endometriosis externa. (47-48) Endometrioid ovarian cancers presents often together with endometrioid endometrial cancer both in an early stage (49, chapter six this thesis)

3: More early stage cancers
Another contrast is that most (80-85%) LS-associated ovarian cancers were early stage cancers (FIGO stage I/II), whereas 60-70% of sporadic and BRCA-associated ovarian cancers are diagnosed as FIGO stage III-IV. (18,27-28,40) Gynaecological surveillance
does not seem to contribute to this earlier diagnosis in LS-associated ovarian cancer when compared to sporadic and BRCA-associated cancers. Therefore, this earlier stage is most probably due to a slower growth pattern and a more favourable biological behaviour. (49-51)

4: Prognosis
The LS-associated ovarian cancers have an overall survival rate of around 80% (12,43,51), which contrasts with sporadic and BRCA1/2 gene mutation-related cases, in which a 10-year survival rate of 20-40% is reported. (16,18,27,51) The more favourable prognosis in LS-associated cases can be explained by the early stage at diagnosis and a different histological type which does not metastasize as often as high grade serous adenocarcinomas in sporadic and BRCA-related ovarian cancers. (49-51)

SUGGESTIONS FOR FUTURE RESEARCH AND CLINICAL ADVICES

1: ENDOMETRIAL SURVEILLANCE IN WOMEN WITH LS

1.1 Counselling during endometrial surveillance
Endometrial cancer in women with LS develops at a mean age of 50-55 years, when most women are perimenopausal. (1-5) At this age, symptoms of irregular or abundant bleeding can be easily misinterpreted by patients and doctors. Therefore, annual surveillance as well as prompt access to interval visits are crucial in LS carriers, especially in the perimenopausal age-range. During the annual surveillance visit women with LS should get adequate counselling about these symptoms, and advice to contact the gynaecologist for an extra surveillance visit including a transvaginal ultrasound and endometrial sampling in case of irregular bleeding at any age, in order to detect an interval endometrial (pre) malignancy as early as possible. Besides, in the study in chapter six, a substantial part of symptomatic women (6/12) seemed to have an ovarian cancer as well as an endometrial cancer. Therefore in symptomatic LS carriers with irregular vaginal bleeding and the suspicion of an endometrial (pre)malignancy one should be aware of synchronous ovarian cancer as well.

1.2 Endometrial sampling during gynaecological surveillance
A study on levels of pain during invasive endometrial sampling during annual
surveillance in women with LS is written in chapter three. In this study 13% of the LS carriers decided for preventive surgery because of (fear for) pain during annual endometrial sampling and 8% of the women refused one or more endometrial samplings because of fear of pain. In women with LS who want to avoid endometrial sampling because of fear of pain, a transvaginal ultrasound only is performed during annual gynaecological surveillance. When the endometrial response is thin (≤ 4 mm) without the presence of any symptoms, standard annual follow-up can be advised without standard endometrial sampling as the additional value of endometrial sampling in these cases is limited (chapter two). When a woman reports irregular bleeding and/or the endometrial response is > 4 mm in postmenopausal women, further examination of the endometrial tissue through endometrial sampling is advised. Women who avoid invasive screening because of fear of pain should be offered the opportunity of an endometrial biopsy or diagnostic hysteroscopy under general anaesthetics or conscious sedation. (chapter three)

1.3 Endometrial surveillance with conscious sedation
From our study it appeared that the burden of a painful annual gynaecological endometrial surveillance is high (chapter three). It also appeared that the value of additional endometrial surveillance in case of normal ultrasonographic findings is limited (chapter two). Nevertheless, endometrial sampling is indicated in case of abnormal ultrasonographic findings or irregular vaginal bleeding. The opportunity of using conscious sedation for short, (< 30 minutes), but painful procedures in hospitals in the Netherlands is increasing at the moment. Gynaecological surveillance including performing an endometrial sampling or a hysteroscopy can be offered in a setting with conscious sedation. This is an alternative for women who reported much pain during annual standard endometrial sampling at the outpatient clinic. However a disadvantage is that this procedure with conscious sedation is more expensive and more time consuming and potentially more risky for side effects of the sedation. (52)

The reported risks of conscious sedation in gynaecological procedures, (as diagnostic and therapeutic hysteroscopies) seems to be low. (52-54) As colonoscopy is performed under conscious sedation for some years now and another option is to perform the gynaecological surveillance including an endometrial sampling (if indicated) in the same setting as the colonoscopy for the surveillance of colon cancer in women with LS. (32-33,55-56) This can be an option for women who reported pain as a reason to refrain from endometrial sampling while having an indication. The other advantage is that gynaecological and colon surveillance can be combined in the same setting, during one visit, which might be more convenient for the patients.
2: OTHER SURVEILLANCE TOOLS DURING GYNAECOLOGICAL SURVEILLANCE IN WOMEN WITH LS

2.1 Endometrial surveillance by using tampons

Given the painful endometrial sampling procedure, other surveillance tools for obtaining endometrial cells in women with LS should be developed. In this thesis a feasibility study of 25 women with LS was performed by using tampons (chapter four). No endometrial cells were found in the cell samples although the obtained epithelial cells and leucocytes were of good quality. It is assumed that most probably, no endometrial cells were found in the tampon samples, because normal endometrial tissue is not shedding and therefore these cells were not found in the vaginal tampons. Other studies found that endometrial cells, shedding in cervical smears, may indicate endometrial pathology especially if vaginal bleeding is present. (57-58) Another way of studying the endometrium by vaginal secretia is by methylated DNA, found in the vagina of women with and without a (pre)malignancy of the uterus. (38-39) These studies concluded that endometrial (pre)malignancies show a higher level of methylation compared to vaginal tampon samples of women with benign endometrium. (38-39)

It will be recommended to perform a large cohort study in women with LS, to evaluate whether (pre)malignant endometrial cells are present in the tampon secretes compared to standard endometrial sampling. Because women with LS have an annual risk of about 2.5% and a lifetime risk of developing endometrial cancer of 15-55% depending on the type of gene mutation, it will be expected to find some (pre)malignancies of the endometrial tissue in a large cohort of women. (1-5,14) Endometrial cells and methylated DNA obtained by the tampons can be collected and compared to the results of standard endometrial sampling. Another possible option will be to evaluate the differences between DNA methylation assays, obtained with tampons in asymptomatic women with LS, who will be diagnosed with endometrial (pre) malignancies, compared to those with normal endometrial tissue. DNA methylation assays might potentially be more sensitive because intact cells are not required, as was needed in the tampon study reported in chapter four. (39)

2.2 Surveillance with liquid biopsies

Another alternative for gynaecological surveillance might be liquid biopsies in blood in the future. The opportunity of detecting cancer by using blood based liquid biopsies in patients with various types of cancer, as non-small cell lung carcinoma, colorectal
cancer, ovarian cancer, melanoma, glioblastoma, pancreatic cancer, hepatobiliary cancer and breast cancer has been evaluated. (59-60) mRNA of blood platelets was analysed and distinguished patients with localized and metastasized tumours from the healthy individuals. The disadvantage of these studies was that none of the patients who were tested had endometrial cancer, all patients had a sporadic type of cancer, most of the tumours were metastasized and circulation tumour DNA was better detectable in metastasized disease. (59-60) Endometrial cancer is mostly found in a pre or an early malignant stage and becomes symptomatic in an early stage. It would be interesting if blood based liquid biopsy is a possible alternative for the current surveillance method consisting of transvaginal ultrasound and endometrial sampling because it will probably improve convenience compared to the current used painful endometrial sampling and might also detect early ovarian and colon cancer. However, these tumour types, (endometrial and ovarian cancer in women with LS), might not be suitable for this procedure because early symptoms mostly leads to the diagnosis of endometrial and ovarian cancer at an early stage in women with LS and metastasis occur lately in these tumours.

3: OVARIAN CANCER SURVEILLANCE IN WOMEN WITH LS

The role of annual gynaecological surveillance to find ovarian cancers in women with LS seems to be limited although there was only information about the surveillance in the minority of patients reported in chapter five and six. The early stage at diagnosis of ovarian cancer (chapter five) could not be attributed to annual gynaecological surveillance because ovarian cancer was also diagnosed in an early stage in patients who did not undergo annual surveillance and were not identified as LS carriers before ovarian cancer diagnosis. Also occult ovarian cancers (in 17-28% of the cases) were reported which were not found during surveillance while performing preventive surgery because of LS. (30,61-63)

A large international cohort study of women with LS, who get annual endometrial and ovarian cancer surveillance might be considered, to formulate a more age- and mutation-specific risk calculation to establish the optimal age to perform surgery and to evaluate the costs and effectivity of annual surveillance versus prophylactic surgery in women with LS.
4. ROLE OF PROPHYLACTIC SURGERY IN WOMEN WITH LS

4.1 Prophylactic surgery for the prevention of endometrial cancer in women with LS

Aim of prophylactic surgery in high-risk patients is to prevent the cancer to develop and/or to prevent the patient to die of the disease. In case of LS-associated endometrial cancer, the incidence is high (15-55%) and the risk of dying in case of the disease is about 15%. (5,8,64-67) The mean age of endometrial cancer in LS is about 50-55 years of age. (3,5,8,64)

Prophylactic surgery for the prevention of endometrial cancer in women with LS can be performed by a laparoscopic procedure during which the uterus (and ovaries and fallopian tubes) are removed, which is effective for the prevention of endometrial (and ovarian) cancer. (68-69) Besides, it is a save and minimal invasive procedure, with a low complication rate. Moreover, there is a lower risk of complications in colonoscopy after laparoscopic surgery when compared to open hysterectomy. (68-75) Nevertheless, doctors should always counsel women about the risks of a surgical procedure consisting of intra operative risks of bladder/urether or bowel injury during surgery and other risks as intra-abdominal adhesions or a vaginal top prolapse after some years. (69,76-79) Especially the intra operative risks may be higher in older women with co-morbidity and after extended abdominal surgery in the past. (74,76,80) Prophylactic surgery can be considered from 45-50 years of age after counselling of the advantages and disadvantages, amongst others premature menopause. (81-82)

4.2 Prophylactic surgery for the prevention of ovarian cancer in women with LS

Aim of prophylactic surgery is also to prevent the cancer to develop and/or to prevent the patient to die of the disease. In case of LS-associated ovarian cancer, the incidence is 6-12% and the risk of dying in case of the disease is about 20%. (14,20,22,25-26,51) These results were also found and described in chapter five and six.

Prophylactic surgery for the prevention of ovarian cancer in women with LS can be performed by a laparoscopic procedure is an effective strategy to prevent ovarian cancer in women with LS and it is a save and minimal invasive procedure. (68,83) The mean age of ovarian cancer in LS is about 40-45 years of age (84) as was described in chapter five and six. It was shown that the age-range of developing ovarian cancer is wide (19-82 years respectively 20-75 years). If preventive surgery is performed to early, more women will suffer from side effects of a sudden onset of surgical menopause.
with impact on sexual functioning and long-term complications from premature menopause. (81-82). If performed too late, more women will suffer from (treatment of) ovarian cancer. The reported risk for the development of ovarian cancer at age 50 is 4.9% and 8.5% at age 70 in all women with LS, with most ovarian cancers diagnosed at FIGO stage I/II with a good prognosis (chapter six).

These characteristics, especially the very young age of development of ovarian cancers in some women with LS, although with a wide age-range, together with a high percentage of early stage cancers and no age-related, mutation specific penetrance curves, makes it difficult to formulate a uniform advise for an optimal age for preventive surgery as is performed in women with BRCA gene mutations. (27,40) Therefore risk reducing salpingo-oophorectomy should be restrained under age 45 because of acute premature menopause and its side effects, although no solid data exist in this patient group. From age 45, preventive surgery can be considered after adequate counselling of the advantages and disadvantages leading to shared decision making with the woman and prophylactic salpingo-oophorectomy should be combined with the prophylactic hysterectomy.

The option of prophylactic hysterectomy and bilateral salpingo-oophorectomy should also be offered to women diagnosed with colon cancer, during the same surgery, from the age of 40 years. (62,85)
REFERENCES


